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13. ABSTRACT *(Maximum 200 Words)*

This project will test the basic hypothesis that a given microsatellite marker allele occurs with greater frequency among the individuals affected with prostate cancer than among the controls. These studies will take advantage of the fact that two populations of Ashkenazi Jewish men are readily available for a case-control study. The first is a group of men at high heritable risk based on their having early-onset prostate cancer. The second is a group of men at low heritable risk who have no personal or family history of prostate cancer. Thus, we expect to observe predisposition alleles in the men at high risk that are not present in the men at low risk. The predisposition genes are likely to be within chromosomal regions in which loss of heterozygosity has occurred. Because these regions have remained identical by descent since the high-risk mutations occurred, they can be recognized by the presence of specific alleles of microsatellite markers in the high-risk group that are not present in the low-risk group.

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INTRODUCTION

This study uses several observations about the genetic basis of prostate cancer to enhance the efficiency of identifying susceptibility genes. 1) Prostate cancer is a multi-step genetic disorder in which some of the observed genetic alterations in prostate cancer cells were acquired through the germline. 2) The chromosomal locations of some of these genes can be identified readily in prostate cancer cells on the basis of their demonstrating loss of heterozygosity. 3) Historically, certain populations have been highly endogamous causing them to have a remarkable degree of genetic homogeneity and to have prevalent founder mutations in some of their disease susceptibility genes. As a result of the population's endogamy, short chromosomal regions have remained identical by descent, leading to recognizable associations of the founder mutations with linked marker alleles (*linkage disequilibrium*). Ashkenazi Jews represent such a population.

BODY

Task 1. Subject identification. Months 1-12

Samples from high-risk subjects have already been identified. The medical histories of each of these subjects have been reviewed, confirming ethnicity and diagnosis of prostate cancer, and noting family history, age of diagnosis and Gleason score at time of diagnosis. For each subject, tissue blocks were obtained for non-cancerous tissues (usually lymph nodes) and thick (50 micron) sections were cut. DNA was purified from these sections using a protocol optimized in our laboratory and then quantified. To extend the utility of these sections, a technique for whole genome amplification using primer extension preamplification (PEP) was optimized. This technique reproducibly provides approximately 50-fold amplification of the DNA samples. From our pool of anonymous low-risk subjects, we have chosen 200 individuals for subsequent analysis. For each subject, the risk profile was determined using a screening questionnaire (figure 1).

Task 2. Development of markers. Months 1-12

A. Markers from regions associated with loss of heterozygosity (LOH) in prostate cancer will be identified and fluorochrome-labeled primers will be synthesized. We have identified microsatellite markers for each of the following chromosomal regions 1q24-q25, 7q31, 8p21-p22, 10q23-q25, 13q14, 16q22, 17p, 17q21-q22, Xq11-q13. Because of uncertainties about relative map positions, we have confined our markers to those which have shown (LOH) in a high proportion of subjects in a single report, to those which show (LOH) in more than one report, or to those whose map positions are known with a high degree of confidence from the GeneMap99 (http://www.ncbi.nlm.nih.gov/GeneMap99) and which are tightly linked to markers that show LOH. In addition, we have added markers for the following chromosomal regions that have shown linkage to prostate cancer susceptibility in families with multiple affected members, 1q24-25, 1q42-43, and Xq27-28 (Smith, et al., 1996, Cooney, et al., 1996, Gronberg, et al., 1997, Xu, et al., 1998, Berthon, et al., 1998).

B. Standard PCR conditions will be developed for each of these markers. The primer sequences for each of these markers was identified using standard databases (http://www.gdb.org). The predicted sizes of the PCR product alleles were noted and markers yielding products of different predicted sizes were grouped and labeled with one of three different fluorescent dyes (tet, fam, hex). The net effect of this grouping is that multiple markers can either be amplified simultaneous and/or pooled from separate amplifications to minimize the number of electrophoretic runs. Procedures for pooling separate amplification reactions have been optimized. (An example of such a pool, including map positions, primer sequences and running conditions for the chromosomal regions 1q24-q25 1q42-q43 is shown in figures 2 and 3).

Different thermostable enzymes were tested for their fidelity for amplifying microsatellites, including AmpliTaq, AmpliTaq Gold, Platinum Taq, Platinum Tsp, and Expand High Fidelity. Among these enzymes, Platinum Tsp (Life Technologies, Gaithersburg, MD) was found to produce the most reliable amplification with the least stutter and the least random addition of an adenine at the 3' end of the PCR product. For each of the markers, different PCR conditions were tested, varying temperature and magnesium chloride concentrations, and the optimum conditions were defined.

C. Individuals with alleles of known sizes will be identified for use in subsequent genotyping analyses. DNA from a non-Jewish female volunteer has been procured. This eliminates the moral dilemma of identifying a potential prostate cancer risk. This DNA has been carried through every optimization, preparative, and analytical step.

KEY RESEARCH ACCOMPLISHMENTS:

Development of high-quality, reproducible methods for microsatellite typing

Development of high-quality, reproducible methods for whole genome amplification

REPORTABLE OUTCOMES:

Proposal, "Genetic Susceptibility to Prostate Cancer in the Netherlands Cohort Study" (PC99-1496), recommended for funding by USARMC

Proposal, "Mentorship Program in Prostate Cancer Genetics" K24 (CA85326-01A1), given a very favorable priority score (146).

CONCLUSIONS

This works demonstrates the feasibility for high-throughput multiplex microsatellite marker analysis and the feasibility for extending small samples of DNA 50-fold for genetic analysis. It creates the foundations for the analyses that will be performed in the remainder of this study.

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APPENDICES

Ostrer, Harry
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NUMBER _____ **FIGURE 1**
FAMILY HISTORY QUESTIONNAIRE YOUR AGE _____

Has any blood relative of yours, i.e. parent, sister, brother, cousin, etc. come to NYU Medical Center for genetic screening?

YES ___ NO ___ DON'T KNOW ____

If yes, what is his/her name? _____Relationship? _____

We would like to obtain some information from you about the occurrence of common diseases in your family. Please read the list below. Check the appropriate box and give the name of the disease where applicable. Include relatives that are both living and deceased.

						Maternal				Paternal			
	Self	Mother	Father	Sister/s	Brother/s	G'Mother	G'Father	Aunt/s	Uncle/s	G'Mother	G'Father	Aunt/s	Uncl e/s
Heart attack (<50 years old)													
Hypertension (High blood pressure)													
Stroke (>60 years old)													
Stroke (<60 years old)													
Breast cancer (<50 years old)													
Breast cancer (>50 years old)													
Ovarian cancer (<50 years old)													
Ovarian cancer (>50 years old)													
Colon cancer (<50 years old)													
Colon cancer (>50 years old)													
Prostate cancer (<50 years old)													
Prostate cancer (>50 years old)													
Malignant melanoma (<50 years old)													
Malignant melanoma (>50 years old)													
ANY other type of cancer (please name)													

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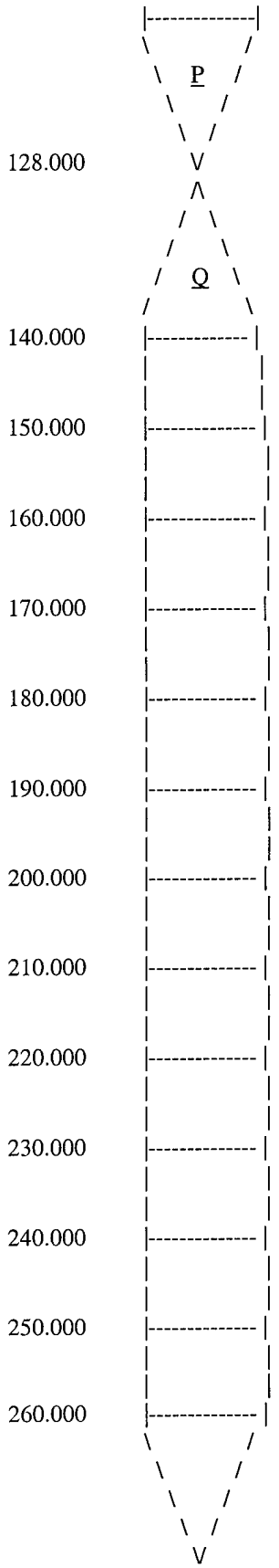
FIGURE 2A*: *MARKERS ON CHROMOSOME 1q24-25
FOR USE IN
PROSTATE CANCER MAPPING STUDY



	WESSEX	GENEMAP '99
1)	D1S2705 (179.995)(0.7600)	
2)	D1S484 (181.700)(0.6400)	2) D1S484 (173.9)(0.6400)
3)	D1S2844 (182.980)(0.8153)	3) D1S2844 (179.2)(0.8153)
4)	D1S2768 (183.333)(0.6132)	
5)	D1S194 (183.518)(0.6669)	
6)	D1S426 (183.520)(0.6059)	
7)	D1S2681 (183.970)(0.7404)	7) D1S2681 (182.8)(0.7404)
8)	D1S196 (185.380)(0.7499)	8) D1S196 (186.4)(0.7499)
9)	D1S431 (185.668)(0.7927)	
10)	D1S2799 (186.018)(0.8800)	
11)	D1S452 (187.510)(0.7506)	
12)	D1S210 (188.290)(0.6454)	
13)	D1S2815 (188.310)(0.8438)	
14)	D1S2790 (188.867)(0.8011)	
15)	D1S218 (189.064)(0.8363)	15) D1S218 (196.5)(0.8363)
16)	D1S2643 (189.648)(0.7506)	
17)	D1S2786 (189.663))(0.7252)	
18)	D1S212 (190.110)(0.7973)	18) D1S212 (198.6)(0.7973)
19)	D1S215 (190.430)(0.7286)	19) D1S215 (199.2)(0.7286)
20)	D1S2640 (190.600))(0.7800)	
21)	D1S2883 (190.601)(0.7545)	
22)	D1S117 (190.902)(0.7940)	
23)	D1S466 (192.109)(0.7709)	23) D1S466 (203.0)(0.7709)
24)	D1S2127 (193.028)(??????)	
25)	D1S158 (193.891)(0.8900)	
26)	D1S254 (194.187)(0.6589)	
27)	D1S518 (194.793)(????)	
28)	D1S222 (194.820)(0.7220)	
29)	D1S238 (195.530)(0.8674)	
30)	D1S428 (197.130)(0.5600) - Possible, but VERY LOW heterozygosity (3)	
31)	D1S422 (197.560)(0.7633)	31) D1S422 (209.4)(0.7633)
32)	D1S2625 (197.960)(0.6100)	
33)	D1S2757 (200.309)(0.8500)	
34)	D1S412 (201.250)(0.7100)	
35)	D1S2816 (203.266)(0.6500)	
36)	D1S413 (204.140)(0.7647)	33) D1S413 (216.5)(0.7647)

Ostrer, Harry
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FIGURE 2B: MARKERS ON CHROMOSOME 1q42.2-43
FOR USE IN
PROSTATE CANCER MAPPING STUDY

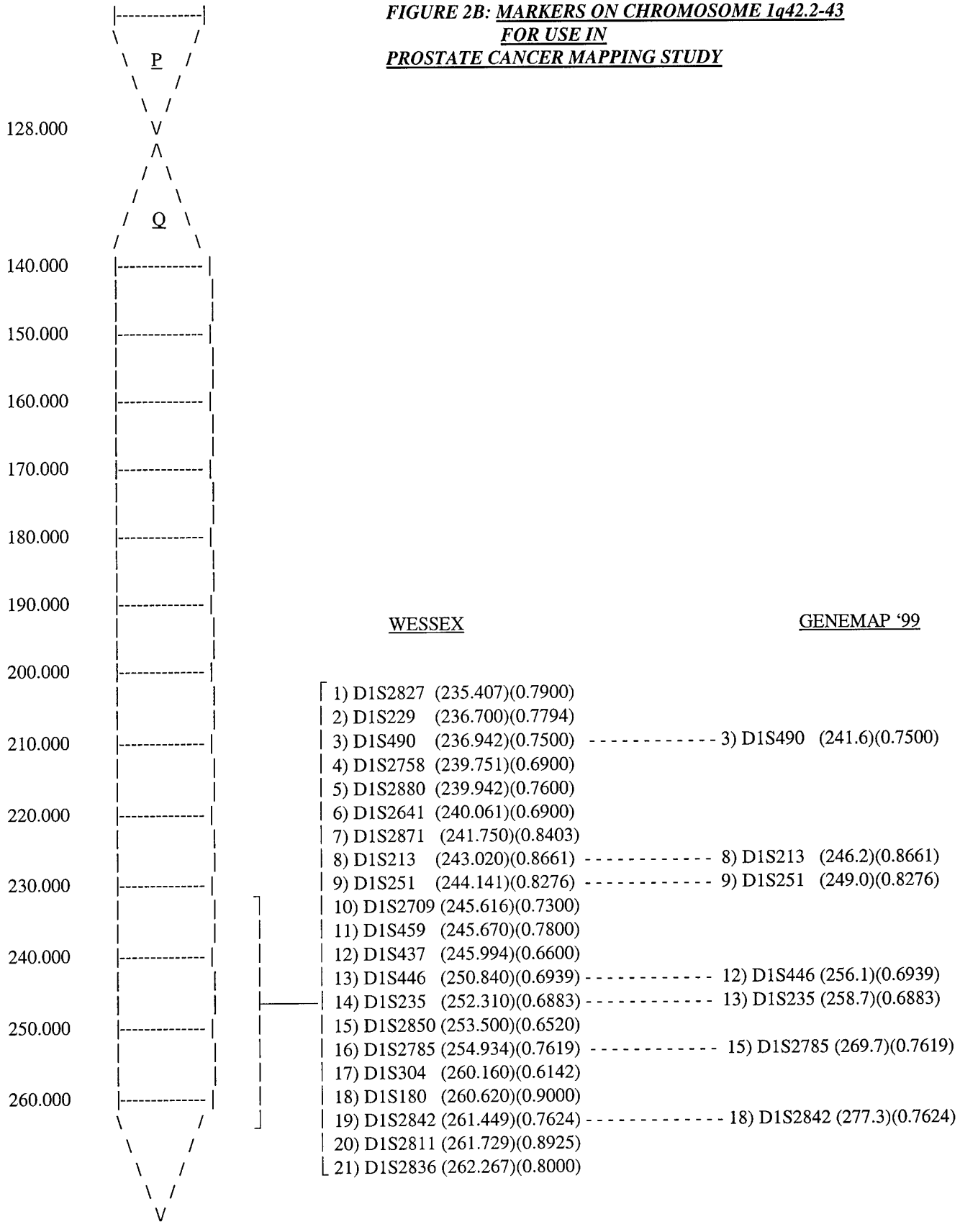

Figure 3

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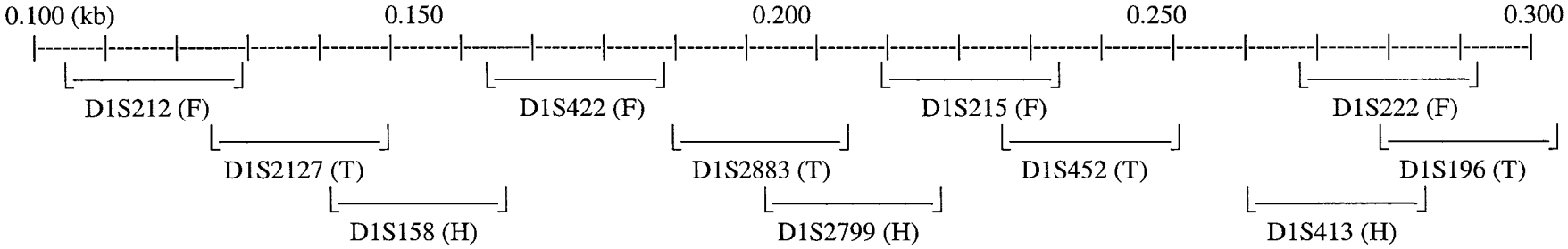
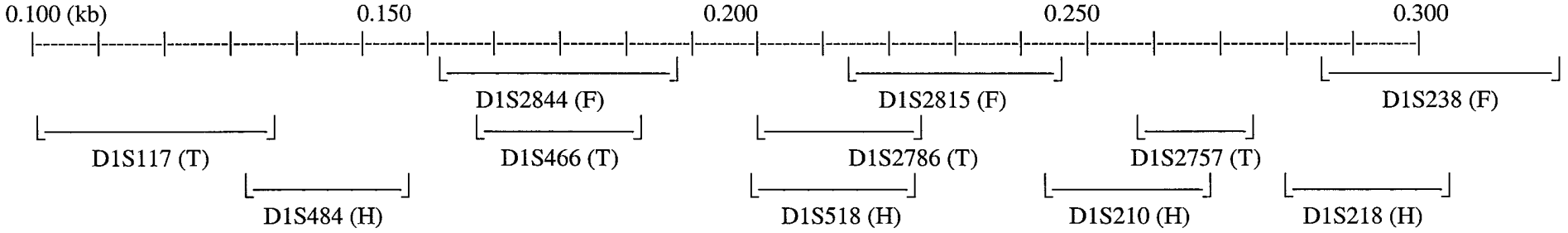
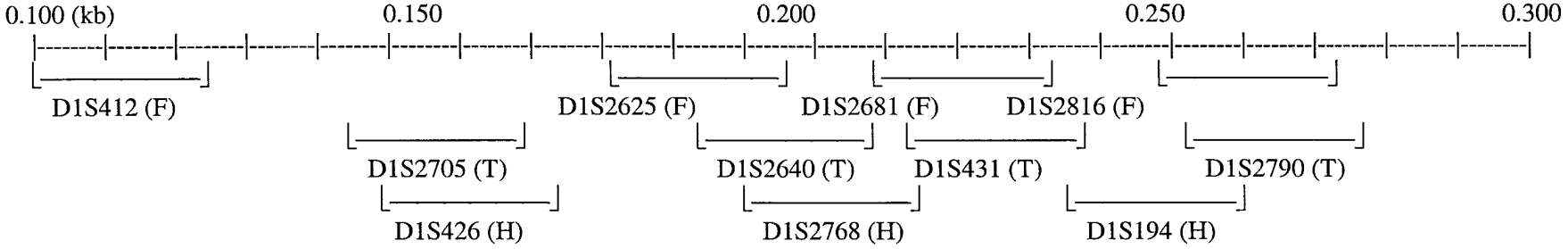
Marker Overlap – Chromosome 1q24-25 - Grouping 1



Marker Overlap – Chromosome 1q24-25 - Grouping 2

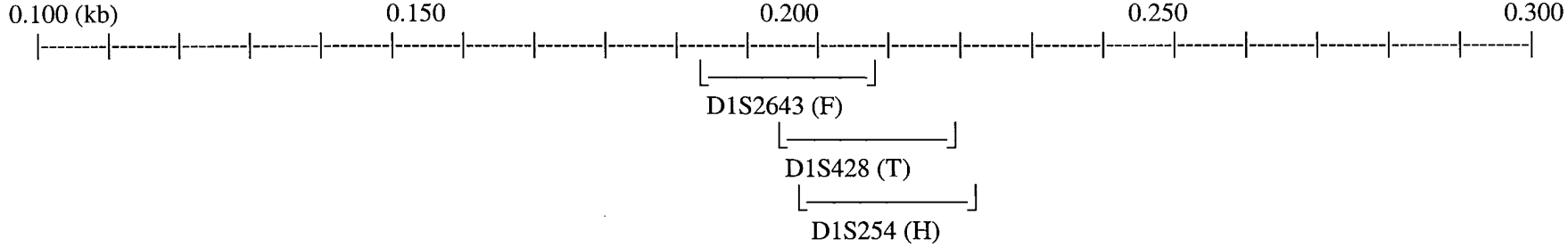


Marker Overlap – Chromosome 1q24-25 - Grouping 3



F = 6-FAM Fluorescent Label T = TET Fluorescent Label H = HEX Fluorescent Label

Marker Overlap – Chromosome 1q24-25 – NEW – High Ranking -



F = 6-FAM Fluorescent Label T = TET Fluorescent Label H = HEX Fluorescent Label

PRIMER SYNTHESIS FOR CHROMOSOME 1q24-25
Grouping 1

Marker Number	Name	Location	Heterozygosity	Size (Min→Max)	Primer Sequence
18	D1S212	190.110 (W) / 198.6 (GM)	0.7973	105→125	F: 5' – FAM – Cag CAA gAC TCT gCC TCT AC - 3'
	Labeled with 6-FAM	Rank = 1			R: 5' – CCA ggC TgA TTT TgT gTA Tg - 3'
24	D1S2127	193.028 (W)	Not Given	123→143	F: 5' - TET – TAA ggg AgA AAA AAA AgC ACC - 3'
	Labeled with TET	Rank = 0			R: 5' – TCT gTT TAT TAA CTA TCT CTC Cag C - 3'
25	D1S158	193.891 (W)	0.8900	137→163	F: 5' – HEX – gggCCT TCT TAT ATT gCT TC - 3'
	Labeled with HEX	Rank = 0			R: 5' – ggA Aag ACT ggA CCA Aag Ag - 3'
31	D1S422	197.560 (W) / 209.4 (GM)	0.7633	158→178	F: 5' – FAM – CAT ggg gTA Tag CAA Cag AC - 3'
	Labeled with 6-FAM	Rank = 1			R: 5' – TgA TTT CCT gCA AAC ATT TT - 3'
21	D1S2883	190.601 (W)	0.7545	179→199	F: 5' – TET – AAA TCT ggT CTT CTg TTT TCA CTAT - 3'
	Labeled with TET	Rank = 0			R: 5' – TTC CAA ATg TTg ACT CTg C - 3'
10	D1S2799	186.018 (W)	0.8800	191 → 209	F: 5' – HEX – AgC Aag ACC CTg TCT CAA AA - 3'
	Labeled with HEX	Rank = 0			R: 5' – Tgg ATA gCT TTC CAC CAC T - 3'
19	D1S215	190.430 (W) / 199.2 (GM)	0.7286	207 →217	F: 5' – FAM – gAC ACA ggT Agg TTA gAA ggA Tg - 3'
	Labeled with 6-FAM	Rank = 1			R: 5' – TgT CTT ggT gAA TTg ACC CT - 3'
11	D1S452	187.510 (W)	0.7506	220 →240	F: 5' – TET – TAA Tgg gTT Cag Tgg ACC TT - 3'
	Labeled with TET	Rank = 3			R: 5' – TgC AgT TCC ATA TTC Cag gT - 3'
36	D1S413	204.140 (W)	0.7647	250 →270	F: 5' – HEX – gCC Aag CCT gAg ATC AAA AT - 3'
	Labeled with HEX	Rank = 1			R: 5' – ACT TgA ACA gAT Tgg gAT Tg - 3'
28	D1S222	194.820 (W)	0.7220	258 → 276	F: 5' – FAM – gCC TTC Tgg CTC TgA AAC TC - 3'
	Labeled with 6-FAM	Rank = 1			R: 5' – CTg Aag AAC CCg CTA TgA Ag - 3'
8	D1S196	185.380 (W) / 186.4 (GM)	0.7499	267→279	F: 5' – TET – ggC TgT ggg TgT TTC TCC TA – 3'
	Labeled with TET	Rank = 2			R: 5' – AgC TCT CAT gNC TTT ACA TTC T – 3'

Ostrer, Harry
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PRIMER SYNTHESIS FOR CHROMOSOME 1q24-25

Grouping 2

Marker Number	Name	Location	Heterozygosity	Size (Min→Max)	Primer Sequence
22	D1S117 Labeled with TET	190.902 (W) Rank = 0	0.7940	100→132	F: 5' – TET – CCT TTT gCC TCC TTC gT – 3' R: 5' – CTC ATT TAC AAT AgC TAC C – 3'
2	D1S484 Labeled with HEX	181.700 (W) / 173.9 (GM) Rank = 3	0.6400	136→142	F: 5' – HEX – AgT gAT gAg ggC CTC TAT TT – 3' R: 5' – AgC TTC TgC CAA CTA TgT gC – 3'
3	D1S2844 Labeled with 6-FAM	182.980 (W) / 179.2 (GM) Rank = 2	0.8153	155→185	F: 5' – FAM – TCC TgA CCT TgC gAT g - 3' R: 5' – Aag Aag TCA CTg AgA ACC Tgg g - 3'
23	D1S466 Labeled with TET	192.109 (W) / 203.0 (GM) Rank = 2	0.7709	160 →180	F: 5' – TET – CAC TgC CTT Tgg ggA C - 3' R: 5' – TCC TgC CTA TCT ggg g - 3'
27	D1S518 Labeled with HEX	194.793 (W) Rank = 0	Not Given	197 →217	F: 5' – HEX – TgC AgA TCT Tgg gAC TTC TC - 3' R: 5' – AAA Aag AgT gTg ggC AAC Tg - 3'
13	D1S2815 Labeled with 6-FAM	188.310 (W) Rank = 2	0.8438	210 → 237	F: 5' – FAM – CTg ACA Tgg AAT ACC TCT ATg ATg C - 3' R: 5' – CTC CAA ATC Tag TCA CAC Tgg AAg - 3'
17	D1S2786 Labeled with TET	189.663 (W) / 197.8 (GM) Rank = 0	0.7252	207→227	F: 5' – TET – CCC TgC TTT CAg TTg gAT A – 3' R: 5' – ggT AgT TCA CAg TCA TTT TTA gAC A – 3'
12	D1S210 Labeled with HEX	188.290 (W) / 193.8 (GM) Rank = 1	0.6454	235→255	F: 5' – HEX – CAC TgA AAA CTT CTT CCC CT – 5' R: 5' – AgC TgA ATC TCA CCC AAT AA – 3'
33	D1S2757 Labeled with TET	200.309 (W) Rank = 0	0.8500	253 → 271	F: 5' – TET – TTT TTT AAT gAC TgA CCA gTg - 3' R: 5' – TgC CTT CTg CTA TgT TTg - 3'
15	D1S218 Labeled with HEX	189.064 (W) / 196.5 (GM) Rank = 0	0.8363	266 → 286	F: 5' – HEX – TgT AAA AgC AAA CTg Tag Acg AT - 3' R: 5' – TTT ATg TTA TCA CCA Agg CTT CT - 3'
29	D1S238 Labeled with 6-FAM	195.530 (W) Rank = 1	0.8674	272 → 302	F: 5' – FAM – TCA TgT CTA gAT CCT gTg CC - 3' R: 5' – Tgg Agg Cag TTT AgA TTg Tg - 3'

PRIMER SYNTHESIS FOR CHROMOSOME 1q24-25

Grouping 3

Marker Number	Name	Location	Heterozygosity	Size (Min→Max)	Primer Sequence
34	D1S412	201.250 (W) / 213.2 (GM)	0.7100	95→115	F: 5' – FAM – TTC CAC AgT CAT TTg AgT CC – 3'
	Labeled with 6-FAM	Rank = 1			R: 5' – TCT CTA gAg AAg CAg AgC CA – 3'
1	D1S2705	179.995 (W) / 175.1(GM)	0.7600	140→160	F: 5' – TET – ggg CgT TTA CCT CTA CAC – 3'
	Labeled with TET	Rank = 0			R: 5' – AAA CAg gCC ACA CTC AAT A – 3'
6	D1S426	183.520 (W) / 181.7 (GM)	0.6059	144→164	F: 5' – HEX – gCA ACC TTC TTA AAC ATg gA - 3'
	Labeled with HEX	Rank = 3			R: 5' – ACC CAA CAT Agg CAT ATC CT - 3'
32	D1S2625	197.960 (W) / 209.9 (GM)	0.6100	175 →195	F: 5' – FAM – gCT CTA ATC ATC CCA CCg C - 3'
	Labeled with 6-FAM	Rank = 1			R: 5' – TCC TCT gAA CTC TCA CAg TgA CTT g - 3'
20	D1S2640	190.600 (W) / 199.7 (GM)	0.7800	182 →202	F: 5' – TET – TgT Tgg AAT gAC CAC CAT A - 3'
	Labeled with TET	Rank = 0			R: 5' – ACT TAA CAC AAT ggC CTg C - 3'
4	D1S2768	183.333 (W) / 176.8 (GM)	0.6132	188 → 208	F: 5' – HEX – ACA CAT TTC CTg CTg gAT Ag - 3'
	Labeled with HEX	Rank = 0			R: 5' – AAg AgC CAT TAC ATC TCT gAA g - 3'
7	D1S2681	183.970 (W) / 182.8.8 (GM)	0.7404	205→225	F: 5' – FAM – AgA CgC ACA TCC ACA gAT AgT ATT – 3'
	Labeled with 6-FAM	Rank = 2			R: 5' – gAC TTg AgA CCC TCA CCA gA – 3'
9	D1S431	185.668 (W) / 187.2 (GM)	0.7927	209→229	F: 5' – TET – CCT AgC ACC TAg Agg CAA – 5'
	Labeled with TET	Rank = 0			R: 5' – ggA ggA TAg CAT ACC AAA AA – 3'
5	D1S194	183.518 (W) / 183.3 (GM)	0.6669	227→ 247	F: 5' – HEX – gTA AgT TTT CTg CTC CAC ATC ATC - 3'
	Labeled with HEX	Rank = 0			R: 5' – CAA TgA ggA CAA TgT CTC TTg CTg - 3'
35	D1S2816	203.266 (W) / 215.2 (GM)	0.6500	240 → 260	F: 5' – FAM – TTC CCC AAA TgT ATT ACT gC - 3'
	Labeled with 6-FAM	Rank = 3			R: 5' – AAA ggA gTA CCC AAT CCC Ag - 3'
14	D1S2790	188.867 (W) / 196.0 (GM)	0.8011	243 → 263	F: 5' – TET – AAA ATg CTC ATT AgT CCA gAA Ag - 3'
	Labeled with TET	Rank = 0			R: 5' – Tgg CTA TgT TTT ACT AgC TCA Ag - 3'

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PRIMER SYNTHESIS FOR CHROMOSOME 1q24-25

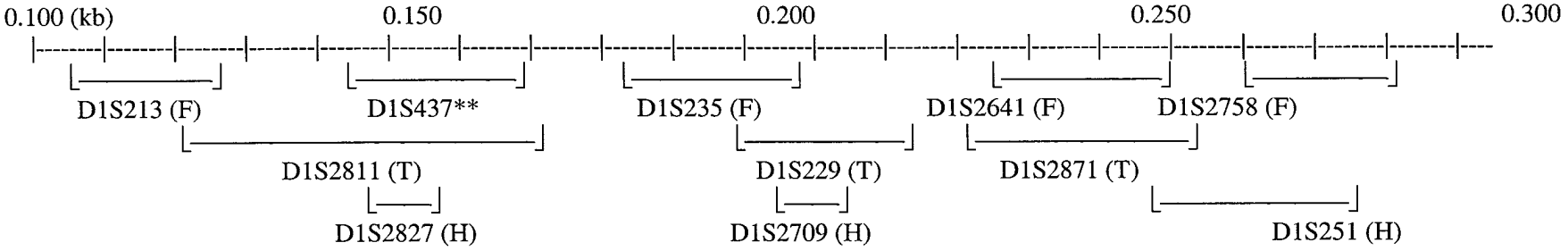
NEW – High Ranking -

Marker Number	Name	Location	Heterozygosity	Size (Min→Max)	Primer Sequence
16	D1S2643	189.648 (W)	0.7506	182→202	F: 5' – FAM – gTg TAT gAT AAA TAA TTT CAg CCC – 3'
	To be labeled with 6-FAM	Rank = 2			R: 5' – CCA TTg gTg CAT TTT gAA – 3'
30	D1S428	197.130 (W)	0.5600	193→213	F: 5' – TET – TCA Tgg ggT AgT gTT gC – 3'
	To be labeled with TET	Rank = 3			R: 5' – Tgg Tgg CCT gTC CAT A – 3'

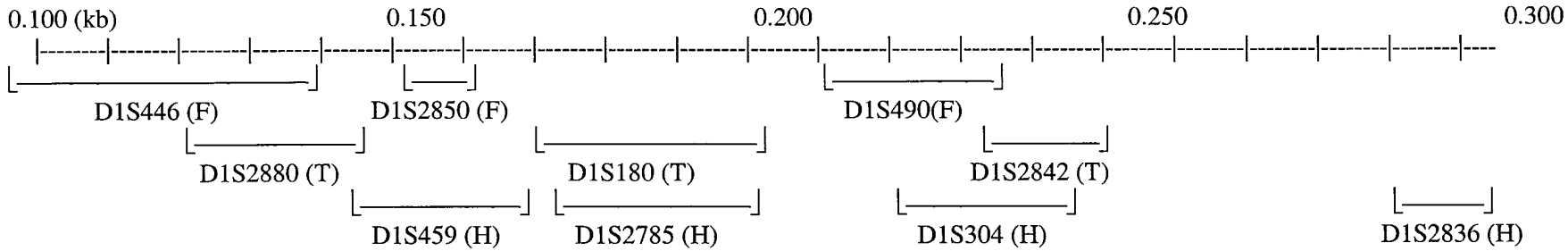
NOTE: The heterozygosity of D1S428 is very low, but the rank is very high.

26	D1S254	194.187 (W)	0.6589	198→208	F: 5' – HEX – ACA ACT TTT ATT TTC CAg gC – 3'
	To be labeled with HEX	Rank = 3			R: 5' – ggA CTC gAT TTA ATC CCA C – 3'

Marker Overlap – Chromosome 1q42.2-43 – Grouping 1



Marker Overlap – Chromosome 1q42.2-43 – Grouping 2



F = 6-FAM Fluorescent Label T = TET Fluorescent Label H = HEX Fluorescent Label

PRIMER SYNTHESIS FOR CHROMOSOME 1q42.2-43

Grouping 1

Marker Number	Name	Location	Heterozygosity	Size (Min→Max)	Primer Sequence
8	D1S213	243.020 (W) / 246.2 (GM)	0.8661	104→124	F: 5' – FAM – CAT TAT CCA Agg TCA ggA gg - 3'
	Labeled with 6-FAM	Rank = 0			R: 5' – AgC TgT TAA TCC AAT CTA TgA TgT g - 3'
20	D1S2811	261.729 (W)	0.8925	120→164	F: 5' – TET – CCA CTg CAC TCC AAC CTg - 3'
	Labeled with TET	Rank = 0			R: 5' – gTA gTT TCT gAC TgA Agg C - 3'
1	D1S2827	235.407 (W)	0.7900	142→152	F: 5' – HEX – gCT TCT ggC CTC TgT CA - 3'
	Labeled with HEX	Rank = 0			R: 5' – AAT TTT gCg TgT gTg TgC - 3'
12**	D1S437	245.994 (W)	0.6600	139→159	F: 5' – FAM – CAg gTg gCC AAA TgT T – 3'
	To be labeled with 6-FAM	Rank = 3			R: 5' – TTT TAT ggC TgA ATA gTA CTC CTT T – 3'
14	D1S235	252.310 (W) / 258.7 (GM)	0.6883	175→195	F: 5' – CAg CAA gAg TTC ATg ggA - 3'
	Labeled with 6-FAM	Rank = 2			R: 5' – AAC AgT CAA TTA CAA AAT ATg TgT g - 3'
2	D1S229	236.700 (W) / 241.6 (GM)	0.7794	188→208	F: 5' – TET – gCT TgT TTC CAT TTA Tgg Tg – 3'
	Labeled with TET	Rank = 0			R: 5' – ACT CTA gTT gTg TgT gAA TgT ATg – 3'
10	D1S2709	245.616 (W)	0.7300	191→197	F: 5' – HEX – TCA TAC CAC ATA TCA gAA TgT C - 3'
	Labeled with HEX	Rank = 0			R: 5' – ATC AAT CAg TAT CTA ATA gCA TCA - 3'
6	D1S2641	240.061 (W) / 242.5 (GM)	0.6900	219→239	F: 5' – FAM – TgC AAg TAg ggT CAg TTT Ag – 3'
	Labeled with 6-FAM	Rank = 0			R: 5' – gCC ATT TAT TTA CTC TgT gTg – 3'
7	D1S2871	241.750 (W)	0.8403	215→241	F: 5' – TET – TgA AgT gTg CAT TCT NTA CAT CA - 3'
	Labeled with TET	Rank = 2			R: 5' – CgA gAC ATT TgC ATC ATC A - 3'
9	D1S251	244.141 (W) / 249.0 (GM)	0.8276	249→271	F: 5' – HEX - gTC TCC AgC CTg CCA C - 3'
	Labeled with HEX	Rank = 0			R: 5' – gAC CAA gCA ACT TCA CTC C - 3'
4	D1S2758	239.751 (W)	0.6900	250→268	F: 5' – FAM – ACA gAg ATT CAC TCT AgT TgC C - 3'
	Labeled with 6-FAM	Rank = 0			R: 5' – TCA ATA TCC Tgg gCT CAA g - 3'

PRIMER SYNTHESIS FOR CHROMOSOME 1q42.2-43

Grouping 2

Marker Number	Name	Location	Heterozygosity	Size (Min→Max)	Primer Sequence
13	D1S446 Labeled with 6-FAM	250.840 (W) / 256.1 (GM) Rank = 1	0.6939	89→132	F: 5' – FAM - TTT CTg ATg ggC Agg g - 3' R: 5' – gTT gTT gCA ggT CTT CAA Ag - 3'
5	D1S2880 Labeled with TET	239.942 (W) / 244.1 (GM) Rank = 0	0.7600	119→139	F: 5' – TET – CgT ggT TCT AAT Cgg C – 3' R: 5' – CAT CAT TTg CTT gCT gC – 3'
11	D1S459 Labeled with HEX	245.670 (W) / 251.2 (GM) Rank = 0	0.7800	138→158	F: 5' – HEX – gAg gAg AgA gAA CCA ATg CT – 3' R: 5' – CTA CAT gTT TCA AgT Tgg CTg – 3'
15	D1S2850 Labeled with 6-FAM	253.500 (W) Rank = 0	0.6520	145→153	F: 5' – FAM – CgA Agg TgT ACT ggg ACT gg - 3' R: 5' – AAT CAg gAT CAT gCT ACA ggg - 3'
18	D1S180 Labeled with TET	260.620 (W) Rank = 1	0.9000	163→189	F: 5' – TET – TCC CTA AAA gAC TgC Aag CT - 3' R: 5' – ACA gAg TCA AAC TgT TgT gg - 3'
16	D1S2785 Labeled with HEX	254.934 (W) / 269.7 (GM) Rank = 0	0.7619	164→187	F: 5' – HEX – CgT gAA TAT CCT CAg ggA AT - 3' R: 5' – ATT gTg gCA CCg TAC TCC - 3'
3	D1S490 Labeled with 6-FAM	236.942 (W) / 241.6 (GM) Rank = 3	0.7500	198→208	F: 5' – FAM – TCC TTA CAA ATg ggA gAC TAC ACA A - 3' R: 5' – Aag ggT TTg AgA Aag TCC TCT ACA - 3'
17	D1S304 Labeled with HEX	260.160 (W) / 272.0 (GM) Rank = 1	0.6142	206→226	F: 5' – HEX – TAT CTC ACT gCA CAg TAT TCC A –3' R: 5' – TTA ggA TAg AAg CTg AAA gCT g – 3'
19	D1S2842 Labeled with TET	261.449 (W) / 0.7624 (GM) Rank = 0	0.7624	217→231	F: 5' – TET – TCA CCT gAC CTg TCC C - 3' R: 5' – Tgg TTC TCA gCC ACA A - 3'
21	D1S2836 Labeled with HEX	262.267 (W) Rank = 2	0.8000	268→281	F: 5' – HEX – TTT AAC CAA ggN ggT gAA Ag - 3' R: 5' – CTg gAA TgA AAT CCT CCC - 3'